New Horizons: Future Directions in Neurotoxicology

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Neurotoxicology is a relatively young discipline that has undergone significant growth during the last 25 years. During the late 1970s and 1980s, numerous national and international conferences and meetings were devoted to the topic of neurotoxicology, the formation of societies or specialty sections related to neurotoxicology, and the establishment of two independent peer-reviewed journals devoted to neurotoxicology. This decade was also associated with a rapid increase in our knowledge of chemical effects on the structure and function of the nervous system. During the 1990s, regulatory agencies such as the U.S. Environmental Protection Agency accepted neurotoxicology as a crucial end point and neurotoxicity testing and risk assessment guidelines were published. Neurotoxicology has also been accepted at the international level as evidenced by environmental criteria documents published by the International Programme on Chemical Safety and testing guidelines by the Organization of Economic Cooperation and Development. In recent years, there has been increased concern that the etiology of some neurodegenerative diseases may be associated with exposure to neurotoxic agents and that subpopulations of humans such as children and the elderly may be differentially sensitive to neurotoxic exposure. In the future, mechanistic information derived from basic research will be used in the identification and characterization of chemicals with neurotoxic potential. *Key words*: future directions, neurotoxicology, neurotoxicology risk assessment guidelines. — *Environ Health Perspect* 108(suppl 3):439–441 (2000).
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In 1980, Reiter (*1*) wrote that neurotoxicologists should move into the mainstream of environmental toxicology by developing a research strategy to evaluate the multitude of chemicals and mixtures in the environment. Ten years later, Tilson (*2*) documented the general growth of the discipline of neurotoxicology as evidenced by the number of national and international conferences, formation of scientific societies or specialty sections, establishment of peer-reviewed journals, and the large increase in the number of scientific papers and books published on topics related to neurotoxicology. In 1990, the Office of Technology Assessment (*3*) published a book reviewing the basic principles and status of neurotoxicology research in the federal government. Acceptance of neurotoxicology at the international level was noted in a publication by the International Programme on Chemical Safety/World Health Organization (IPCS/WHO) of an environmental criteria document (*4*) on the principles and methods for assessment of neurotoxicity associated with exposure to chemicals.

Significant progress was made in three areas during the 1980s to address the concern raised by Reiter (*1*), i.e., the development of a research strategy to assess the large number of chemicals in the environment. One important development was the general acceptance of behavioral procedures in neurotoxicological studies. Prior to the 1980s, it was generally accepted that chemical-induced changes in the structure of the nervous system were adverse, whereas changes in behavior were

not universally accepted as evidence of neurotoxicity. Determination of the sensitivity and selectivity of behavioral changes became an important issue, since it was argued that such changes might precede neuropathological changes and provide a more sensitive indicator of a chemical's neurotoxicity. Mello (*5*) was among the first to argue that the behavior of organisms represents a functional integration of the nervous system and that nervous system capacity cannot be assessed in neurohistological or physiological studies independent of behavioral analyses. On this basis, it was argued that behavioral measures have significant potential in the study of deleterious effects of chemicals on the nervous system (*6*). In the 1980s there was a large increase in the number of studies using behavioral procedures to investigate the effects of chemicals on the nervous system. Regulatory acceptance of behavioral tests in neurotoxicological assessments became evident with the development of neurotoxicity testing guidelines by the U.S. Environmental Protection Agency (U.S. EPA) (*7*), many of which include behavioral end points.

A second development was the evolution of tiered testing strategies in which each stage of evaluation incorporates decision points as to whether available information is sufficient for determining the neurotoxicity of a chemical (*8*). For example, Evans and Weiss (*9*) outlined a three-tier testing scheme, including hazard identification, characterization, and assessment of human susceptibility. First-tier tests include neurological screening

batteries, cage-side observations, and measures such as motor activity and grip strength. Neuropathological observations may also be used in the first tier in conjunction with the functional tests. If a chemical was observed to be neurotoxic in the first tier, a decision to characterize the chemical, i.e., move to the second tier, would have to be made. Characterization studies might be based on results from the first tier, already existing published data, or on new toxicological data suggesting that the chemical may pose a human neurotoxic risk. Second-tier tests are designed to focus on specific aspects of chemical-induced neurotoxicity. For example, a second-tier test might be used to determine effects of a chemical on cognitive function such as attention or sensory function such as visual acuity. Evans and Weiss (*9*) also suggested a third tier to assess human susceptibility to chemicals, using methods analogous to those employed in animal studies. In 1992, the National Research Council (NRC) (*10*) published a book on environmental neurotoxicology describing a three tier-testing scheme similar to that of Evans and Laties (*6*) but included mechanistic rather than human studies in the third tier. A three-tier testing strategy was recently endorsed by the European Chemical Industry Ecology and Toxicology Centre (*11*). The relative limitations of a tier-testing strategy within a regulatory context, however, have been noted by Tilson et al. (*8*).

A third development in the late 1970s and 1980s that addresses Reiter's (*1*) concern for a research strategy was the standardization and validation of methods. In 1978, Tilson and Cabe (*12*) noted the general lack of test

validation in animal models and suggested a strategy aimed at resolving this problem. These investigators proposed that test validation of animal models be accomplished by evaluating known neurotoxicants in a battery of tests chosen to assess effects reported in humans. By comparing the observed results of the neurotoxicants in the animal models with the predicted effects, investigators could make decisions concerning the validity of selected tests. This approach was used to validate the National Toxicology Program behavioral screening battery (13,14). Interlaboratory studies to standardize and validate tests for developmental neurotoxicology were reported by Kimmel et al. (15), whereas an international collaboration on neurobehavioral screening methods was completed only recently (16). A number of standardized test batteries now exist for initial assessment of chemicals for potential neurotoxicity (17).

In summary, the 1980s brought a greater acceptance of behavioral techniques in neurotoxicological studies. In addition, a large increase occurred in the number of studies reporting the effects of chemicals on the nervous system. Although these studies added greatly to our knowledge about which chemicals affect nervous system integrity, many were not mechanistically driven. Many test methods were also developed, standardized, and validated, which helped lead to development of neurotoxicity testing guidelines and routine use of neurotoxicological end points in hazard identification.

Neurotoxicology in the 1990s

In 1990, Tilson (2) identified several research gaps in neurotoxicological research. For example, research was needed to develop, validate, and interpret biological markers of exposure and effect for use in humans. Biomarkers are early indicators of variation in cellular or biochemical components or processes—structures of functions that are measurable in a biological system or sample. Many papers were published in the 1990s describing the effects of chemicals on structural and functional end points, and many of these meet the definition of a biomarker of effect. For example, chemically induced injury to the central nervous system may be accompanied by hypertrophy of astrocytes, and in some cases, these astrocytic changes can be seen at the light microscopic level with immunohistochemical stains for glial fibrillary acidic protein (GFAP), the major intermediate filament protein in astrocytes. GFAP has been proposed as a marker of astrocyte reactivity or as a response of the nervous system to injury. The interpretation of chemical-induced increases in GFAP as a biomaker of neurotoxic effect can be augmented by corroborative results from neuropathology, and

measures of GFAP are now included in the neurotoxicity screening battery of the U.S. EPA (7).

An example of a commonly accepted biomaker of exposure is plasma acetyl-cholinesterase (AChE) activity. Organophosphate and carbamate pesticides inhibit the activity of AChE, which is an enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). Inhibition of AChE prolongs the action of ACh in the synaptic cleft and is associated with a range of cholinomimetic effects produced by these compounds. Decreases in plasma AChE are now generally accepted as a biomarker of exposure to organophosphate and carbamate pesticides. However, such changes are not always associated with the presence of clinical signs of cholinergic overstimulation, so they are not regarded as a biomarker of neurotoxic effect. Identification of other biomarkers of neurotoxic effect and exposure for hazard identification and risk assessment remains a high priority for future research. Such biomarkers may also be useful in the development of biologically based dose–response models.

Tilson (2) also noted that *in vitro* models for neurotoxicity assessment should be used with greater frequency in the 1990s. This prediction was based on the need to screen large volumes of agents for potential neurotoxicity in a cost-effective and timely manner and the relative success of *in vitro* techniques in other areas of toxicology hazard identification. The possibility of using *in vitro* techniques for the routine screening of neurotoxicity was recently addressed by an IPCS work group (18). They pointed out that *in vitro* procedures generally do not take into account distribution of the toxicant in the body, route of administration, or metabolism of the substance. Furthermore, they noted the difficulty in extrapolating *in vitro* data to many animal or human neurotoxicity end points, including behavioral changes, motor disorders, sensory and perceptual dysfunction, and cognitive deficits. The group emphasized, however, that *in vitro* systems are well suited to study biological processes in more isolated conditions and have been used successfully to understand mechanisms of toxicity, identify target sites of action, and characterize the cellular and molecular changes induced by exposure to neurotoxicants. Harry et al. (18) conclude that *in vitro* tests have their greatest potential in providing information on basic mechanistic processes to refine specific experimental questions to be addressed in the whole animal. Therefore, a battery of *in vitro* tests selected for the ability to detect specific mechanisms of neurotoxicity or sites of effect might eventually be developed for neurotoxicology hazard identification.

Tilson (2) also notes that research is needed to clarify the role that environmental factors appear to play a role in the etiology of some neurodegenerative diseases. For example, several neurodegenerative diseases such as amyotrophic lateral sclerosis–Parkinsonism–dementia complex, neurolathyrisms, and mussel poisoning have been associated with excitatory amino acid-induced neuronal damage (19), whereas exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine has been shown to produce a Parkinson's-like syndrome in humans and experimental animals (20,21). More recent research has implicated young-onset Parkinson's disease (22) and extrapyramidal disorders (23) to exposure to pesticide agents. Clearly, this is an important area for future research, since some neurodegenerative diseases appear to have a genetic component that could be affected by environmental influences.

It is now widely recognized that human environmental exposure to chemicals is not associated with a single chemical (24). Because exposure may occur either simultaneously or sequentially to large numbers of agents from different sources or by differing routes, there are few commonly accepted approaches for the risk assessment of mixtures. Tilson (2) noted the need to determine if the neurotoxicity of individual chemicals differs quantitatively and qualitatively from that of the same chemicals in a mixture. Some experiments with mixtures of neurotoxic agents suggest that they act in an additive or less-than-additive fashion. For example, Rebert et al. (25) exposed rats by inhalation to pairs of solvents that cause hearing damage when given individually. Hearing loss was evaluated using electrophysiological techniques and the effects were predicted by a linear dose-additive model, indicating an additive rather than a synergistic or antagonistic interaction. Kodavanti and Ward (26) studied the interactive effects of several polychlorinated biphenyl (PCB) congeners *in vitro*. Their results also suggest that the biological effects of mixtures of PCB congeners fit a dose-additive model. It is clear that understanding the toxicology of chemicals in mixtures is a complex problem, and better mechanistic information will be needed to predict synergistic effects.

Tilson (2) also pointed out that neurotoxicology risk assessment would grow during the 1990s. During this decade, significant progress has been made to develop and validate methods to screen and characterize all classes of neurotoxicants, to better understand structure–activity relationships for several classes of chemicals, to improve extrapolation from animal data to human risk, and to characterize neurotoxic mechanisms for some chemicals. This progress s indicated by the

publication of the U.S. EPA neurotoxicology risk assessment guidelines (27), which describe the principles, concepts, and procedures that the agency uses in evaluating data on potential neurotoxicity associated with exposure to environmental toxicants. However, significant issues for neurotoxicology risk assessment remain, including the use of mechanistic data in risk assessment calculations, development of more quantitative risk assessment models, and determination of adequate protection for susceptible subpopulations such as infants, children, and the elderly.

In summary, significant progress was made during the 1990s in many of the research areas identified by Tilson (2), including biomarkers, use of *in vitro* techniques in neurotoxicology, chemical mixtures, environmentally associated neurodegenerative disorders, and neurotoxicology risk assessment. However, significant research gaps remain, particularly in our understanding of neurotoxic mechanisms, the etiology of neurodegenerative diseases, and the behavior of chemicals in mixtures.

Neurotoxicology in the New Millennium

The discipline of neurotoxicology continues to grow and evolve. Research to elucidate mechanisms of neurotoxicity and neurodegenerative disease is needed to identify potential environmental causes of environmental disease and to develop possible treatments. In the area of hazard identification, research is needed to develop and validate short-term *in vitro* tests or batteries of tests that can detect potential neurotoxicants on the basis of mechanism of action. Indeed, mechanistic data are needed to construct and validate biologically based dose–response models that can be used in neurotoxicology risk assessment.

Future research will continue to focus on mechanisms underlying differential responsiveness to chemical exposure by various subpopulations. For example, research is needed to provide comparative pharmacokinetic data for possible sensitive populations and to identify genetic contributions underlying differential responsiveness to chemical exposure.

Much future research will continue to focus on the problems of children and infants. For example, there is a need to elucidate the functional modalities that may be altered following developmental exposure and to develop improved animal models to examine the neurotoxic effects of exposure during the premating and early postmating periods and in neonates. Researchers need to better understand the relationship between maternal and developmental neurotoxicity and to provide information concerning the concept of a threshold for certain types of developmental neurotoxicological effects.

The toxicological assessment of chemical mixtures remains a very complex problem. Research is needed to address mechanisms of synergistic or antagonistic response of chemicals given together via the same or differing exposure media. Additional research is needed to improve animal models for examining the effects of agents given by various routes of exposure and determine the effects of recurrent exposures over prolonged periods of time. Such research will aid in the evaluation and interpretation of data obtained from real-world environmental exposures and will lead to methods to assess risk more precisely.

Finally, research is needed to advance the application of more quantitative models in neurotoxicology risk assessment. Approaches for improved mathematical modeling of neurotoxic effects need to be developed if neurotoxicological data are to be used routinely in risk assessment.

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